CONSTRUCTION OF LOW DENSITY LIPOPROTEIN/FOLATE RECEPTOR CHIMERAS TO CHARACTERIZE HOW MEMBRANE ANCHORS TARGET RECEPTORS TO COATED PITS AND CAVEOLI. H Matsue*, RGW Anderson*, KG Rothberg*, PR Bergstresser, BA Kamen, SW Lacey. UT-Southwestern, Dallas, TX

The entry of low density lipoproteins (LDL) into cells begins by specific binding to LDL-receptors (LDL-R) in coated pits. Reduced folates are also internalized by a specific receptor but the folate receptor (FR) clusters in non-coated pits called caveoli. Our laboratory previously showed that the folate receptor is anchored by glycosyl-phosphatidylinositol (GPI). We hypothesized that differential localization of LDL-Rs and FRs in coated pits and caveoli is dependent on their respective anchors. We therefore designed chimeric LDL/folate receptors with swapped anchor sequences. Two chimeras and two controls were constructed using a PCR mutagenesis method. The LDL-R ectodomain was attached to the GPI anchor signal of the FR to make an LDL-R chimera that binds LDL but has a GPI anchor. The FR chimera was constructed using a similar method that apposed the ectodomain of the FR to the anchor domain of the LDL-R. Wild type LDL-R and FR controls were constructed to ensure that proteins with the same anchor also have identical 3' untranslated regions. COS-1cells transfected with each construct were examined by immunofluorescence. Each of the four constructs expressed a clustered surface protein recognized by the appropriate antibodies. The clustering patterns of the LDL-R chimera and wild type folate receptor were similar while the folate receptor chimera and wild type LDL-R shared a pattern. A CHO line that doesn't express FRs or LDL-Rs was transfected to make lines expressing each construct. Immunofluorescence examination again demonstrated that all four constructs express a surface protein that clusters. The immunofluorescence pattern of wild type FRs and LDL-Rs are different. The immunofluorescence patterns for the chimeric FR and wild type LDL-R are similar. Likewise, the immunofluorescence patterns of the chimeric LDL-R and wild type FR are indistinguishable. This suggests that the anchors for the FR (GPI) and LDL-R participate in directing expression of receptors to caveoli and coated pits respectively.

COMPLEXITY OF ANTIBODY BINDING EPITOPES ON HLA-DR MOLECULES. X Fu* and RW Karr, VA Medical Center and the Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA.

In previous studies, we found that several antibody (Ab) binding epitopes on DR4 or 7 molecules are determined primarily by residues within amino acids 1-40 of DR4 or 7β1 chains. The structural basis for polymorphic Ab epitopes on DR molecules was further investigated using flow cytometry to analyze binding of monoclonal antibodies (mAb) to transfectants expressing wild-type, mutant, or hybrid DRβ chains with DRα. Studies of epitopes on DR4 or 7 molecules indicate that residue 4 of the DR4 (Arg) or DR7β1 (Gln) chain is both necessary and sufficient for epitopes recognized by several mAb. Single amino acid substitutions at DRβ position 4 in molecules that normally bind the mAb caused loss of binding, and substitutions at these positions in molecules that do not bind the mAb caused a gain of binding. Studies of epitopes on DR4 molecules recognized by 4 other mAb indicate that DR4β1 25 Arg is necessary for these epitopes, but the epitope is not created by a 25 Gln → Arg substitution in DR7β1. Binding data with molecules containing hybrid DR4/7β1 chains, which subdivide the chains into segments of amino acids 1-20, 21-40, 41-97, and the β2 domain, indicate that a polymorphic residue, perhaps 14, in the 1-20 segment is also required for these epitopes. Binding of other mAb to molecules containing hybrid DR1/5β1 or DR5β1/DR5β3 chains indicate that residues in the 41-97 segment of DRβ chains are the primary determinants of epitopes recognized by most of these mAb. The binding patterns of some mAb suggest critical contributions to epitopes from residues in both the 1-40 and 41-97 segments. These data demonstrate that multiple residues in DRβ chains, some of which are not predicted to be accessible to Ab in the current model of a class II molecule, are critical for Ab binding epitopes.

SOMATOSTATIN ANALOG STIMULATES INTESTINAL MIGRATING MOTOR COMPLEX ACTIVITY AND REDUCES BACTERIAL OVERGROWTH IN PATIENTS WITH PROGRESSIVE SYSTEMIC SCLEROSIS. H. Soudah*, W. Hasler*, C. Owyang. Dept. of Int. Medicine, University of Michigan, Ann Arbor, MI.

We have previously demonstrated that somatostatin stimulates intestinal motility. In this study we examined the effects of the long-acting somatostatin analog octreotide on duodenal motility and symptomatic bacterial overgrowth in progressive systemic sclerosis (PSS) patients with pseudoobstruction to define a therapeutic role for octreotide. Basal motility was recorded via perfused intestinal catheters for 4 hrs in 5 PSS patients with bacterial overgrowth documented by breath hydrogen (H_2) >20ppm after 50gm oral glucose and in 6 normal volunteers. Stimulated motility was recorded for 3 hrs after octreotide injection. Octreotide 10μg sq increased the number of duodenal phase III complexes from 1.5±0.4 to 4.1±0.5 per 3 hrs in normals. In PSS patients, who had no spontaneous phase III activity, octreotide (100μg sq) induced 3.6±1.4 complexes/3 hrs which propagated at the same velocity as spontaneous complexes in normals. Areas under the pressure curves of individual phase IIIs were 2/3 as great in PSS patients as in normals (36.5±8.4 vs 57.8±3.1). We examined the effects of chronic octreotide therapy on symptoms and breath H_2 in the PSS patients. Octreotide 50μg sq qhs x 3 wks decreased fasting breath H_2 from 25.1±3.0 to 3.9±1.3ppm (p=0.002) and decreased peak H_2 after 50gm oral glucose from 74.5±13.3 to 14.0±4.1ppm (p=0.013). Symptoms were scored daily (0=none, 1=mild, 2=moderate, 3=severe symptoms). 3 wks of octreotide decreased nausea scores from 1.8±0.7 to 0.2±0.1, bloating scores from 2.9±0.1 to 0.6±0.2, and abdominal pain scores from 2.1±0.3 to 0.6±0.2 (all p<0.05). Stool frequency increased from 0.7±0.4 to 2.2±0.6 movements/day. In conclusion, octreotide stimulates propagative duodenal phase III activity in PSS patients with pseudoobstruction. Chronic octreotide therapy reduces bacterial overgrowth and improves pseudoobstructive symptoms. Thus octreotide may be a useful prokinetic agent for PSS patients with intestinal dysmotility.

DIFFERENTIAL EXPRESSION OF PHOSPHOLIPASE C-γ1 AND C-γ2 IN LYMPHOCYTE POPULATIONS AND CELL LINES. RD Goldfien, *TF Gajewski, *D Qian, *J Weber, *FW Fitch, and JB Imboden. Dept. of Medicine UC San Francisco, and Dept. of Pathology Univ. of Chicago.

Phosphatidylinositol-specific phospholipase C (PLC) comprises a large family of related enzyme isoforms that mediate signal transduction through the production of soluble inositol phosphates (InsP) and diacylglycerol. Two PLC-γ isoforms have been described: in fibroblasts PLC-γ1 is tyrosine phosphorylated and is activated by growth factor receptor tyrosine kinases. A PLC-γ2 cDNA was first isolated from a transformed B cell, and PLC-γ2 message is abundant in spleen, but its function in lymphocytes and other cells is unknown. We have confirmed that PLC-γ2 message is highly expressed in unfractionated mouse splenic lymphocytes. Isolated splenic T cells and thymocytes, however, express abundant PLC-γ1, but little if any PLC-γ2 message. We have also studied 2 murine T helper 2 clones which fail to produce InsP in response to antigen stimulation (D10 and PL3). These clones express little or no detectable message for either PLC-γ1 or PLC-γ2. In contrast, 2 T helper 1 clones (PGL-2 and GL18), which do produce InsP upon activation, express abundant message for PLC-γ1 but not PLC-γ2. We conclude that 1) There is differential expression of PLC isoforms among various lymphocyte subpopulations and cell lines; 2) T cells and thymocytes appear to preferentially express PLC-γ1 message and; 3) some, but not all murine T helper clones express PLC-γ1 and expression of PLC-γ1 may correlate with the ability of these clones to generate InsP in response to antigen stimulation. These results suggest that the PLC-γ1 isoform may be responsible for TCR-mediated InsP production.

IMMUNOLOGY II

Subspecialty Session

Sunday, May 5, 1991
2:30 PM

Room 604

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF A COMMON AFRICAN ALLELE OF THE CD4 MOLECULE. S. Lederman, JA DeMartino*, BL Daugherty*, MJ Yellin*, I Foeldvari*, AM Cleary*, GE Mark* and L Chess. Columbia University, NY, NY and Merck & Co., Rahway, NJ.

The CD4 molecule is a relatively non-polymorphic 55 kDa glycoprotein that plays important roles in immune physiology and as a receptor for the Human Immunodeficiency Virus. An allele of CD4 present in nearly 30% of people of African descent has been identified by non-reactivity with the monoclonal antibody, OKT4 and evidence has been presented to suggest that individuals who are homozygous for this phenotype are predisposed to autoimmune disease and may independently have abnormal T-B interactions. The genetic basis for the OKT4- polymorphism of CD4 is unknown. In the present paper, the structure of the CD4 molecule from an homozygous CD4/OKT4- individual was characterized at the molecular level. The size of the CD4/OKT4- protein and mRNA were indistinguishable from those of the OKT4+ allele. The polymerase chain reaction (PCR) was used to map the structure of CD4/OKT4- cDNAs by amplifying overlapping DNA segments and to obtain partial nucleotide sequence after asymmetric amplification. PCR was then used to clone CD4/OKT4- cDNAs spanning the coding region of the entire, mature CD4 protein by amplification of two overlapping segments followed by PCR recombination. The nucleotide sequence of CD4 cDNA clones revealed a G->A transition at bp 867 encoding an R->W substitution at amino acid 240 relative to CD4/OKT4+. Expression of a CD4/OKT4- cDNA containing only this transition in transient assays on transformed human kidney (293) cells and on stably transfected CD4- Jurkat D1.1 cells, confirmed that the R->W substitution at amino acid 240 ablates the binding of the mAb OKT4. The location of the R->W substitution is in the membrane proximal, V3 domain that is known not to be involved in either interacting with Class II MHC molecules or the coat protein (gp120) of HIV. The CD4/OKT4- expressing Jurkat D1.1 were compared to CD4/OKT4+ Jurkat D1.1 cells for their ability to express CD25 (tac, p55 of IL-2R) after stimulation with immobilized OKT3 (anti-CD3/T cell receptor complex) and for their ability to activate resting B cells to express CD23. No difference was detected between CD4/OKT4- and CD4/OKT4+ in these assays. The observation that CD4/OKT4- molecules were functionally indistinct from CD4/OKT4+ is consistent with the location of the amino acid substitution in a membrane proximal domain. However, the V3 region of CD4 may be involved in horizontal interactions with ligands confined to the same membrane such as the T cell receptor and an alteration in this type of interaction might conuribute to the immune abnormalities associated with this allele.